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Toxicity of Dietary and Intravenously Administered Moniliformin to Broiler Chickens[1]

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ABSTRACT Moniliformin (1-hydroxy-cyclobut-1-ene-3,4-dione), either purified (0, 8, and 16 mg/kg of diet) or from culture of *Fusarium moniliforme* strain NRRL 6322 on corn grits (8, 16, and 64 mg/kg of diet) was fed to growing broiler chicks from 1 to 21 days of age. Up to 16 mg moniliformin/kg of diet from either source was without effect on chick weight gain, feed consumption, and mortality. Chicks fed 64 mg moniliformin/kg of diet from culture had reduced weight gain and feed consumption. Total daily moniliformin consumption by these chicks was nearly twice the reported single oral 50% lethal dose. Three of 10 chicks fed 64 mg/kg of moniliformin in the diet died. No lesions were found upon necropsy. The LD_{50} of purified moniliformin upon intravenous injection of 7-week-old female broiler chickens was 1.38 ± .035 mg/kg body weight. Average time to death was 65 minutes. Progressive symptoms noted included lack of muscular coordination, tachypnea from moderate to severe followed by slow labored respiration, coma, terminal agonal struggle, and death.

(Key words: moniliformin, mycotoxin, broiler chicken, LD_{50}, *Fusarium moniliforme)*

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INTRODUCTION

Moniliformin (1-hydroxy-cyclobut-1-ene-3,4-dione) is a mycotoxin produced by *Fusarium moniliforme* and *F. fusaroides* which are found commonly on corn and other crops (Cole *et al.*, 1973; Kriek *et al.*, 1977). Burmeister *et al.* (1979) reported an LD_{50} of 5.4 mg/kg body weight upon oral intubation of day-old chicks. The corresponding LD_{50} for intraperitoneal injected mice was 29.1 mg/kg for males and 20.9 mg/kg body weight for females. In both mice and chicks, animals became recumbent within 4 to 6 hr and died within 24 hr. Kriek *et al.* (1977) found an LD_{50} of 3.68 mg/kg in ducklings and 50.0 and 41.6 in male and female rats. Feeding *F. moniliforme* culture containing moniliformin to rats resulted in acute congestive heart failure. Symptoms noted in animals have generally been described as muscular weakness, respiratory distress, cyanosis, coma, and death.

Recently, Burmeister *et al.* (1980) noted that mice could tolerate quantities of moniliformin exceeding their acute LD_{50} dosage when the toxin was administered in drinking water over a 24 hr period. Thiel (1978) has shown that moniliformin is a potent inhibitor of the pyruvate dehydrogenase enzyme complex and suggests this as the primary mechanism for its toxicity.

Studies of effects of moniliformin in chickens have been confined to intubations of day-old chicks. The current studies were undertaken to examine the effects of both pure moniliformin and culture containing moniliformin on performance of chicks to 3 weeks of age. Additionally, the acute toxicity of injected moniliformin in 7-week-old birds is reported.

EXPERIMENTAL PROCEDURE

Moniliformin (Na form) was extracted and purified from a culture of *F. moniliforme* strain NRRL 6322 on corn grits as described by Burmeister *et al.* (1979). Analysis of the purified moniliformin by UV absorption at 229 nm and atomic absorption spectroscopy for sodium indicated 98% purity. The *F. moniliforme* culture on corn grits fed to chicks contained 15 mg moniliformin/g of culture by analysis. Analysis was by dilution and comparative thin layer chromatography, using

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quenching of the fluorescent indicator by moniliformin on Silica Sel 60 F-254 plates (Burmeister *et al.*, 1979).

Broiler type chickens (Ross males × Arbor Acre females) were used in these studies. In the first experiment, weighed quantities of moniliformin were dissolved in a small amount of water, mixed with corn, and amended to the nutritionally complete broiler diet (Chi *et al.*, 1977) to provide levels of 0, 8, and 16 mg moniliformin per kilogram of diet. The *F. moniliforme* culture on corn grits was substituted for corn into this same diet to provide 8, 16, and 64 mg moniliformin per kilogram of diet. Day-old chicks (mixed sex) were housed in electrically heated batteries (35 C) with raised wire floors. Feed, water, and light were provided continuously during the 21 day assay period. Feed consumption and body weights were determined weekly. Dead chicks during the experiment and all chicks at the end of the experiment were necropsied for lesions.

For the intravenous injection study, 7-week-old female broiler chicks that had been reared on the same diet as used in the previous study were weighed, injected *via* the wing vein with appropriate quantities of moniliformin in .85% saline (2 mg moniliformin/ml), and returned to cages. Symptoms and time to death were recorded. Survivors were maintained on the same diet for 10 days, weighed, and necropsied for lesions. Preliminary studies (data not shown) indicated the appropriate range of dosages.

Data were subjected to analysis of variance and treatment means were compared using Duncan's multiple range test (Steel and Torrie, 1960). The LD_{50} of intravenously injected moniliformin was estimated by the method of Miller and Tainter (1944).

RESULTS

Purified moniliformin was without effect on chick weight gains, feed consumption, and mortality (Table 1). The one chick that died on 16 mg moniliformin/kg diet apparently had an internal infection as evidenced by a swollen abdominal cavity filled with yellowish fluid. Only the highest level of moniliformin containing culture (64 mg/kg of diet) resulted in reduced chick weight gains ($P<.05$). All 3 mortalities noted with chicks fed 64 mg/kg moniliformin from culture had no lesions upon necropsy. These birds were growing normally and had feed in their crops and digestive tracts at time of death. All surviving chicks were normal upon necropsy. Gross examination for leg deformities proved negative.

Intravenous injection of moniliformin produced a rapid, acute death (Table 2). Of the birds injected, only one bird was noted to have visible symptoms of toxicity and subsequently recover. The estimate of the LD_{50} for moniliformin is 1.38 ± .035 mg/kg of body weight. Weight gains of birds surviving the single intravenous dose of moniliformin were unaffected by dosage level.

The clinical symptoms of birds that died from injection of moniliformin can be divided into four stages as follows: 1) apparent lack of apprehension, close eyes slowly and repeatedly,

TABLE 1. *Body weight gain, feed consumption, and mortality of broiler chicks fed moniliformin for 3 weeks*

Dietary moniliformin	Body weight gain	Feed consumption	Mortalities
(mg/kg of diet)	(g/chick)		(No.)
0	519[a]	800	0
8	543[a]	859	0
16	566[a]	860	1
8[1]	524[a]	801	0
16[1]	556[a]	834	0
64[1]	447[b]	777	3
Pooled SE	27.2	34.0	

[a,b]Two groups of 10 mixed sex chicks were fed each experimental diet from 1 to 21 days of age. Means with different superscript letters differ ($P<.05$).

[1] From unextracted culture of *Fusarium moniliforme* grown on corn grits. This culture contained 15 mg moniliformin/g of culture.

TABLE 2. *Acute toxicity of moniliformin administered intravenously to female broiler chickens*[1]

Dosage moniliformin	Mortality/number	Minutes to death[2]	Body weight gain of survivors[2]
(mg/kg body weight)			(g/10 days)
1.0	0/2	. . .	400 ± 127
1.2	0/3	. . .	473 ± 12
1.3	4/8	70 ± 45.6	435 ± 118
1.4	1/8	62	393 ± 72
1.5	4/4	61 ± 28.6	. . .

[1] Moniliformin was administered *via* wing vein in .85% saline solution containing 2 mg/ml moniliformin. Average initial weight of the birds was 1246 g.

[2] Mean ± standard error.

moderate tachypnea, legs forward and spraddled, restlessness, sitting down forcefully; 2) extremely lethargic, tail bobs up and down due to severe tachypnea, open mouth respiration; 3) bird down in the cage and prostrate, very slow, irregular, labored respiration; 4) coma, terminal agonal struggle, death.

DISCUSSION

Moniliformin up to 16 mg/kg of diet either in culture or purified was without effect on 3 week chick performance. In contrast to the report of Sharby *et al.* (1973), this culture of *F. moniliforme* caused no increase in leg abnormalities. Cause of death of chicks given 64 mg/kg of diet of moniliformin from culture could not be ascertained, but it seems probable that sufficient toxin was consumed to cause death similar to that noted in the intravenous injection study. The daily consumption of moniliformin for birds fed moniliformin at 64 mg/kg of diet averaged 9.7 mg/kg of body weight over the 3 week assay period. This is considerably in excess of the single acute LD_{50} dosage of 5.4 mg/kg body weight estimate of Burmeister *et al.* (1979) for day-old chicks. In agreement with the findings of Burmeister *et al.* (1980) with mice, animals appear to be able to tolerate considerably more moniliformin when administered over an extended time period than when administered in a single acute dosage. Further support of this is that the LD_{50} for intravenous injection is 1.38 mg/kg of body weight as compared to 5.4 mg/kg of body weight for oral intubation. This argument, of course, neglects possible inefficient absorption of moniliformin given orally.

It appears that even though moniliformin is extremely toxic to chicks they can tolerate reasonable quantities in their feed. However, data are lacking on levels of moniliformin that may be found in nature, and until such information is available it is impossible to assess the true significance of this mycotoxin to animal health.

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